RESEARCH ARTICLE

Low-Level Laser Therapy: An Experimental Design for Wound Management: A Case-Controlled Study in Rabbit Model

Background: There is a wide array of articles in medical literature for and against the laser effect on wound healing but without discrete effect determination or conclusion. This experimental study aims to evaluate the efficacy of low-level laser therapy on wound healing. **Materials and Methods:** Thirty-four rabbits were randomly enrolled in two groups after creating a full thickness of 3 × 3 cm wound. The intervention group received low density laser exposure (4 J/cm^2) on days 0, 3 and 6 with diode helium-neon low-intensity laser device (wl = 808 nm) and in control group moist wound dressing applied. Finally, wound-healing process was evaluated by both gross and pathological assessment. **Results:** Fibrin formation was the same in the two groups ($P = 0.4$) but epithelialisation was much more in laser group ($P = 0.02$). Wound inflammation of the laser group was smaller than that of the control groups but statistical significance was not shown ($P = 0.09$). Although more smooth muscle actin was found in the wounds of the laser group but it was not statistically significant ($P = 0.3$). Wound diameter showed significant decrease in wound area in laser group ($P = 0.003$). **Conclusion:** According to our study, it seems that low-level laser therapy accelerates wound healing at least in some phases of healing process. So, we can conclude that our study also shows some hopes for low level laser therapy effect on wound healing at least in animal model.

KEYWORDS: Experimental study, low level laser therapy, wound healing

INTRODUCTION

Wound healing is one of the most challenging problems in today's medical issues especially in surgery field. For decades different methods for wound-healing acceleration and prophylaxis against infection has been suggeste.[1,2] Beside chemical materials, some physical techniques such as electro-stimulation of wound, negative pressure wound therapy (NPWT) and also low-level laser therapy (LLLT) are suggested.[3] There is a wide array of articles in medical literature for and against the laser effect on wound healing[4] but without discrete effect determination or conclusion. In this study, we reviewed previous articles and also studied the effect of low-intensity laser in comparison to classical daily irrigation method on wound healing in rabbit model.

Access this article online	
Quick Response Code:	Website: www.jcasonline.com
	DOI: 10.4103/0974-2077.129962

MATERIALS AND METHODS

In an animal model study, 34 male rabbits of the same race were randomly evaluated in two groups. This study was done in animal laboratory of Shiraz University of Medical Sciences, with permission of research ethics committee. All the rabbits received the same care and food during this study. In all rabbits, a full thickness of 3 × 3 cm wound was made with a surgical scalpel via a transparent measured tool. The intervention group received low density laser exposure (4 J/cm^2) on days 0, 3 and 6 with diode helium-neon low-intensity laser device, wl = 808 nm, Eufton, Italy. In this group after each session, wound dressing was done with sterile gauze that was sutured in the wound area. In the control group, the wound was irrigated with sterile normal saline solution, and wound dressing was done in the same way till crust formation. All the wounds were evaluated on days 0, 3, 6, 13 and 20 with the same

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transparent standardised instrument. For controlling of the observer bias, measurement of the wounds was done by three blinded observes from the Department of Surgery. The Mean of wound measurement of these three observers was applied in the final analysis. Digital photographs were made of the all wounds using the same camera, identical settings and lighting in each session. At the end of study, on the day 20, under general anaesthesia wound biopsy was taken for microscopic and immunohistochemistry (IHC) evaluation. The slides were reviewed by the same pathologist and grading of epithelialisation, inflammation and fibrosis was reported as grading from 1 + to 3+. Also, smooth muscle actin (SMA) reported with IHC method. During the study period, oral and intravenous antibiotics were not applied. For statistical methods in a blind manner in SPSS version 13, rational data were compared with Kruskal-Wallis test. Wound measurement was compared with repeated measure ANOVA. *P*-value less than 0 .05 was reported significant.

RESULTS

During the study period, one rabbit of the control group died on the first day, and four rabbits in this group died on days 13-20. In intervention group, three rabbits died on days 13-20. Autopsy of all died rabbits ruled out wound infection in the two groups. Changes of wound area of died rabbits were calculated in the final analysis. In this study, three plus wound epithelialisation was shown in 38.6% of the laser group vs. in 0 percent of control group ($P = 0.02$) [Figure 1]. Fibrin formation was the same in the two groups ($P = 0.4$). Wound inflammation of the laser group was smaller than that of the control groups but statistical significance was not shown ($P = 0.09$). Also, more SMA was found in the wounds of the laser group but it was not statistically significant ($P = 0.3$) [Figure 2]. Table 1 displays nominal data that were compared between the two groups. Changes of wound diameters showed significant decrease in wound area in laser group during study period ($P = 0.003$) [Table 2].

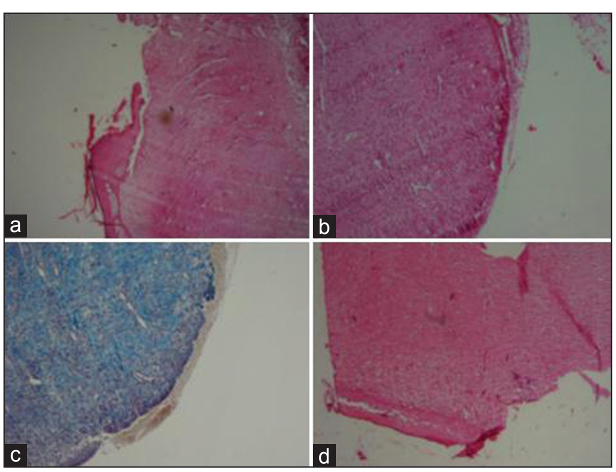

Figure 1: Histological sections of different stages of wound-healing process in study group: a) ulcer, (b) granulation tissue formation, (c) fibrosis, (d) scar formation

DISCUSSION

As mentioned in the introduction section, there is a wide array of studies on the effect of laser therapy on wound healing in comparison to previous methods and its affect assessment grossly and histopathologically. In clinical studies, many investigators have found contradictory results of the effects of LLLT on wound healing. In laboratory animals accelerated wound closure, increased wound epithelialisation and improved tensile strength of scars were seen.[5-8] Many other studies showed no improvement of the healing process through LLLT and

Table 1: Comparison of pathologic finding between two groups

P-value	Laser group *n* (%)	Normal saline group *n* (%)	Grading	Parameters
0.003	5 (%35/7)	9 (%75)	+	Epithelialization
	5 (%35/7)	3 (%25)	++	
	4 (%38/6)	0 (%0)	+++	
0.09	11 (%78/8)	5 (%41/7)	+	Inflammations
	0	2 (%16/7)	++	
	3 (%22/4)	5 (%41/7)	+++	
0.4	2 (%16/6)	1 (%8/3)	+	Wound fibrin
	8 (%66/6)	6 (%50)	++	
	4 (%33/3)	5 (%41/7)	+++	
0.3	5 (%35/7)	6 (%50)	+	SMA
	4 (%28/6)	6 (%50)	++	
	5 (%35/7)	0	+++	

Table 2: Comparison of wound area (cm²) changes during study period

NS mean ± SD	laser mean ± SD	N (Intervention/control)	Parameters (Day)
10/1±1/4	10±2	17/17	Before intervention
8/7±1/4	8/4±1/7	17/16	3
7/1±0/96	6/7±1/7	17/16	6
5/2±1/6	5/9±1/1	17/16	13
4/8±1/2	3/3±1/1	14/12	20

$P = 0.003$

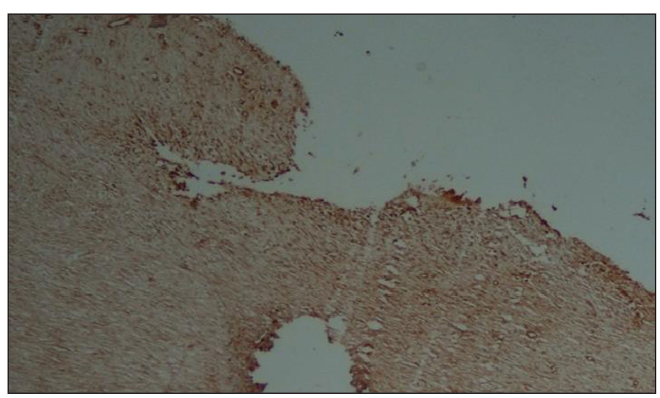

Figure 2: IHC for smooth muscle actin

the above-mentioned affects could not be reproduced. According to experimental studies, low-level laser radiation activates individual cells via three principle effects:

1. The photobiological action mechanism via activation of the respiratory chain: Primary photoacceptors are terminal oxidases as well as NADH-dehydrogenase.
2. Activation of other redox chains in cells: In phagocytic cells irradiation initiates a nonmitochondrial respiratory burst (production of reactive oxygen species, especially superoxide anion) through activation of NADPH-oxidase located in the plasma membrane of these cells. The irradiation effects on phagocytic cells depend on the physiological status of the host organism as well as on radiation parameters.
3. Indirect activation of cells via secondary messengers released by directly activated cells: Reactive oxygen species produced by phagocytes, lymphokines and cytokines produced by various subpopulations of lymphocytes, or NO produced by macrophages or as a result of NO-haemoglobin photolysis of blood cells.[9]

Also, *in vitro* data suggests that LLLT facilitates collagen synthesis[10], keratinocyte cell motility[11], and growth factor release.[12] and transforms fibroblasts to myofibroblasts.[13] However, the benefits of LLLT in wound healing are still controversial. Some animal studies has supported the use of LLLT to facilitate wound healing.[14,15] Conversely, several studies have shown no advantage in healing with LLLT.[16-19] These conflicting results are likely due to variations in treatment factors and limitations in experimental design, including comparison of heterogenous clinical wounds, lack of control groups and limited or no blinding of investigators.[20,21]

Although, there are human studies that have shown that LLLT is effective in burn scar healing[22] and superficial wound healing[23], Atabey *et al*. studied on effect of helium-neon laser on wound healing in rabbits and on human skin fibroblasts *in vitro* but their data did not show significant benefit.[24] In a recent study, the effects of topical tripeptide copper (TTC) complex and helium-neon laser on wound healing has been evaluated in rabbits in which clinical and histopathological effects of TTC and two different doses of laser application (helium-neon laser, 1 and 3 J/cm^2) on wound healing with untreated control wounds is compared.[25] In this study, the median time for the first observable granulation tissue was shorter in the low and high-dose laser groups than in the control group, but was not different between the TTC and control groups. Filling of the open wound to skin level with granulation tissue was faster in the TTC and high-dose laser groups than in the control group, but was not different between the low-dose laser and control groups. The average time for healing was shorter in the TTC and high-dose laser groups, but was not different between the low-dose laser and control groups. Histopathologically, wound healing was characterised by a decrease in the neutrophil counts and an increase in neovascularisation. The TTC and high-dose laser groups had greater neutrophil and vessel counts than in the control group, suggesting a more beneficial effect for wound healing. But Kana *et al*. has shown that the maximum effect of LLLT is with an energy density of 4 J/cm^2.[26] In another study, Nunez *et al*. assessed He-Ne laser effects on blood microcirculation during wound healing with laser Doppler flowmetry *in vivo*.[27] They found out flow alterations and inflammatory response. There were no statistical differences between the groups and the results did not show any significant sustained effect on microcirculation with helium-neon in their study. Gál *et al*. showed that LLLT at 670 nm positively influenced all phases of rat skin wound healing by studying both irradiated and control wounds.[28] Some studies such as Moore *et al.* revealed the effect of laser wave length on cell proliferation response and showed that fibroblast proliferate was faster than endothelial cells in response to laser irradiation and most proliferation response occurs in 665 and 675-nm light.[29] So as discussed above the effect of LLLT on wound healing is very controversial. In our study with low-level laser (wl = 808 nm with 4 J/cm^2 of wound) as mentioned in the results, effect of laser therapy on wound healing assessed both clinically and histopathologically. Our clinical assessment of wound healing was done in double blinded gross assessment method which confirmed statistically better wound healing in laser group in comparison to control group. But according to our pathological findings, just accelerated epithelialisation of the wound, as the effect of LLLT, was found to be statistically significant. Therefor, according to our study it seems that low-level laser therapy accelerates wound healing at least in some phases of healing process. Hence, we can conclude that our study also shows some hopes for low-level laser therapy effect on wound healing at least in rabbit model.

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How to cite this article: Hodjati H, Rakei S, Johari HG, Geramizedeh B, Sabet B, Zeraatian S. Low-level laser therapy: An experimental design for wound management: A case-controlled study in rabbit model. J Cutan Aesthet Surg 2014;7:14-7.

Source of Support: Nil. **Conflict of Interest:** None declared.